Citation: Ferrario, J.; Byrne, C.; Lorber, M.; Saunders, P.;Leese, W.; Dupuy, A.; Winters, D.; Cleverly, D.; Schaum, J; Pinsky, P.; Deyrup, C.; Ellis, R.; Walcott, J. 1997. A statistical survey of dioxin-like compounds in United States poultry fat. presented at Dioxin '97, the 17th International Symposium on Chlorinated Dioxins and Related Compounds, held August 25-29 at Indianapolis, USA. Short paper in, Organohalogen Compounds, Volume 32: 245-251.

Posting of short paper approved by Ecoinforma Press, Jean-Paul-Str. 30, D-95444 Bayreuth. Fax: 49-021-54626. E-Mail: otto.hutzinger@uni-bayreth.de

A Statistical Survey of Dioxin-Like Compounds in United States Poultry Fat

Joseph Ferrario¹, Christian Byrne¹, Matthew Lorber², Pat Saunders³, William Leese³, Aubry Dupuy¹, Dwain Winters⁴, David Cleverly², John Schaum², Paul Pinsky², Cindy Deyrup³, Richard Ellis³, Jim Walcott³

¹Environmental Chemistry Laboratory, Office of Prevention, Pesticides, and Toxic Substances, United States Environmental Protection Agency, Stennis Space Center, Mississippi, 39529.

² National Center for Environmental Assessment, Office of Research and Development, United States Environmental Protection Agency, Washington, D.C., 20460. ³Food Safety and Inspection Service, United States Department of Agriculture, Washington, D.C., 20250. ⁴Office of Prevention, Pesticides, and Toxic Substances, United States Environmental Protection Agency, Washington, D.C., 20460.

1. INTRODUCTION

The paper reports on the results of a joint survey of the United States Department of Agriculture (USDA) and the United States Environmental Protection Agency (EPA) on the rate of occurrence and concentration of chlorinated dibenzo-p-dioxins (CDDs), chlorinated dibenzo-furans (CDFs), and coplanar polychlorinated biphenyls (PCBs) in the fat of U.S. poultry animals. This survey is the first statistically designed national survey of levels of CDDs/CDFs/PCBs in poultry animals in the U.S. It was prompted by EPA's Reassessment of Dioxin-Like Compounds¹ and funded from EPA's Dioxin Exposure Initiative². It is the third joint USDA/EPA effort of its kind. The first two were national studies of beef back fat³ and pork belly fat⁴.

This report has been developed and reviewed by representatives from both EPA and USDA, but has not been externally peer reviewed.

2. SURVEY DESIGN

The primary objective of this survey was to assess the national prevalence and concentrations of polychlorinated dibenzo-p-dioxins (abbreviated CDDs), polychlorinated dibenzo-furans (CDFs), and dioxin-like coplanar polychlorinated biphenyls (coplanar PCBs, or just PCBs) in the abdominal fat of poultry animals slaughtered in federally inspected establishments in 1996. The first step in meeting this objective was to characterize the poultry industry in the United States. This information was used as the basis for developing a sampling frame of establishments that slaughter poultry, and for designing a statistically based (probability) sampling plan.

The four poultry classes are: young chickens, light fowl, heavy fowl, and young turkeys. These are the only individual classes which account for 0.5% or more of the poultry slaughter, and together, these classes account for 99.6% of all poultry slaughtered at federally inspected establishments. Other poultry classes with total slaughter less than 0.5% of the total include, for

example, duck, geese, old turkeys, and rock cornish game hens. In the year between 6/95 and 5/96, USDA reported that over 7.8 billion poultry animals were slaughtered in approximately 252 federally inspected establishments. Table 1 provides a breakdown of the total number of poultry animals slaughtered at federally inspected establishments by slaughter class. This table also shows the number of samples obtained from each slaughter class to comprise the final sample size of 80.

Establishment specific slaughter information from the Food Safety and Inspection Services's (FSIS) Animal Disposition Reporting System (ADRS) was used to construct sampling frames for each of the four slaughter classes, and to randomly select establishments to participate in the survey. There are approximately 178, 17, 12, and 45 federally inspected establishments currently slaughtering young chickens, light fowl, heavy fowl, and young turkeys, respectively. For each slaughter class, the sampling frame included only the largest establishments which account for 99.5% of all animals slaughtered annually. The smallest establishments were excluded since they slaughter only a small number of poultry per week and may not have had an animal available for sampling during the short time period of the sampling.

The survey design called for three-bird composite abdominal fat samples to obtain a total of 80 samples. A composite was required in order to insure that enough abdominal fat sample was available for analysis. The 80 samples were divided as: 41 young chicken samples, 12 light fowl samples, 12 heavy fowl samples, and 15 young turkey samples. The latter three classes were oversampled in order to optimize the ability to distinguish concentration patterns among the four classes, and to allow for an estimate of the variability of the slaughter class estimates. The majority of samples, 41, covered the young chickens which account for almost 95% of total poultry production. The sample size was determined by resource constraints on laboratory analyses of CDDs, CDFs, and coplanar PCBs. Abdominal fat was selected because it was a matrix that was very high in lipid content (in the range of 60-90% lipid), and therefore, the ability to measure the dioxin-like compounds was maximized.

In order to achieve a random sample, the selection was performed in two stages. The first stage was to randomly select a federally inspected slaughter establishment from the sampling frame, and the second was to have the USDA inspector at the establishment randomly select the three animals for the composite sample. For each animal class, establishments were selected from a sampling frame with probabilities in proportion to the total number of those animals slaughtered at the establishment as provided in the ADRS. The final sample set of 80 originated from 70 establishments. Approximately one half of a pound of abdominal fat was obtained from three animals. The samples were collected on a randomly selected day and time within a short (3-4 weeks) time period in September/October of 1996.

3. LABORATORY ANALYSIS

EPA's Environmental Chemistry Laboratory (ECL) at Stennis Space Center, Mississippi, extracted, prepared, and analyzed the samples. The procedures used to analyze the poultry samples were similar to the procedures used to analyze for these compounds in beef fat. These procedures for beef fat are described in Ferrario, et al.⁵ for CDDs/CDFs, and in Ferrario, et al.⁶ for the coplanar PCBs. Sample analysis was based on a modified version of USEPA Method 1613: Tetra-through Octa-chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, April 1990. The percentage of lipids in the tissue samples was determined by lipid determination procedures described in EPA Method 8290: Polychlorinated Dibenzodioxins (PCDDS) and Polychlorinated Dibenzofurans (PCDFS) by High-Resolution Gas Chromatography/High-Resolution Mass Spectrometry (HRGC/HRMS). The abdominal fat samples averaged 80% lipids. Samples were analyzed for the seventeen CDD/CDF compounds which have toxicity equivalency to 2,3,7,8-TCDD and the dioxin-like coplanar PCBs 77, 105, 118, 126, 156, 157, and 169. The CDD/CDFs and the coplanar PCBs

were separated using carbon column chromatography and analyzed separately.

Samples were ground and homogenized, fortified with ¹³C recovery surrogates, and solvent extracted. The extracts were cleaned using a combination of acidified and basic silica gel, alumina, and carbon column chromatography. The final extracts were reduced to volume and spiked with an internal standard prior to analysis by HRGC/HRMS.

Replicates of the abdominal fat matrix were spiked at approximately the lowest expected method quantitation limits for the seventeen 2,3,7,8 substituted dioxins and furans. From an examination of the resulting data, the mean recoveries, standard deviations, the percent relative standard deviation (% RSD) were confirmed, and the target Limits of Quantitation (LOQs) were calculated. The target Limits of Detection (LODs) were estimated to be one half of the target LOQs. The target LODs/LOQs are for CDDs and CDFs in the abdominal fat matrix, which averaged 80% lipid. Therefore, to calculate a lipid-based LOD/LOQ for each sample, these limits need to be divided by the lipid fraction of the specific sample:

	TCDD/F	PCDD/F	HxCDD/F	HpCDD/F	OCDD/F
LOD, ppt	0.05	0.25	0.25	0.25	0.50
LOQ, ppt	0.10	0.50	0.50	0.50	1.00

The LODs and LOQs for the coplanar PCBs were determined based on background laboratory levels as determined by examining the method blanks and from the evaluation of samples spiked at the LOQ. A further discussion of the issue of background laboratory levels of the coplanar PCBs, and the application of that information to the measurement of these compounds in beef fat, can be found in Ferrario, et al.⁶. The final reported levels in the abdominal fat were determined by first analyzing the abdominal fat and subtracting the background concentrations from the concentrations measured in the matrix:

	PCB 77	PCB 118	PCB 105	PCB 126	PCB 156	PCB 157	PCB 169
LOD, ppt	0.80	30.0	10.0	0.10	4.00	1.00	0.08
LOQ, ppt	0.80	30.0	10.0	0.10	4.00	1.00	0.08

4. RESULTS

All results were adjusted to the lipid content of the sample by dividing the whole weight concentration (ppt) in the sample by the lipid fraction in each sample. The lipid-adjusted concentrations were then converted to the 2,3,7,8-TCDD toxic equivalence (TEQ) using the International-Toxic Equivalence Factor (I-TEFs) scheme⁷ for CDD/CDFs and the WHO recommendations for coplanar PCBs⁸. Results were generated assuming that non-detects were equal to 1/2 the limit of detection, and also assuming that non-detects equal 0.0. National extrapolations were not performed for this abstract; all data are summaries of raw data only.

There were two poultry samples, both from the young chicken class, which stood out from all others. The congener concentrations of these two samples are shown in Tables 2 and 3 compared against a summary of the congener concentrations of the other young chickens as well as the other three poultry classes. Table 2 summarizes this information for the dioxins and furans, and Table 3 presents this information for the coplanar PCBs. It can be seen that the two elevated samples had

significantly higher concentrations of all the dioxin congeners, by one to greater than two orders of magnitude, as compared to all other groupings. However, the furan and coplanar PCB congeners from these two samples are quite comparable to all other groupings of poultry.

The impact of these two samples on the estimated mean TEQ and mean 2,3,7,8-TCDD levels in the young chicken class is considerable. For the full population of the young chicken class (n=41), the mean TEQ concentrations would be 1.77 pg/g lipid (1.56 pg/g lipid when ND = 0) and the mean 2,3,7,8-TCDD concentration would be 1.03 pg/g lipid (1.02 pg/g lipid when ND=0). Separating out the two high concentration young chicken samples from the full young chicken class would yield means of 24 pg TEQ/g lipid for the 2 high samples and 0.64 pg TEQ/g lipid (0.41 pg TEQ/g lipid when ND=0) for the 39 other samples. Similarly, separating out the two high concentration young chicken samples from the full young chicken class would yield means of 18 pg 2,3,7,8-TCDD/g lipid for the 2 high samples and 0.16 pg 2,3,7,8-TCDD/g lipid (0.15 pg/g lipid when ND=0) for the 39 other samples. Dixon and Grubs outlier tests¹⁵ at the 95% confidence level were performed the 2,3,7,8-TCDD concentrations found in the 2 high samples. The results from both tests indicate that the values can be considered statistical outliers.

The TEQ concentration and the concentrations of individual congeners for the 78 other poultry samples in this survey are comparable to concentrations reported for poultry in the literature, and also comparable to concentrations of these compounds reported in beef and pork. As seen in Tables 2 and 3, the class average CDD/CDF TEQ concentrations ranged from about 0.40 to 0.99 pg/g lipid for the four classes of poultry and the PCB TEQ concentrations ranged from 0.28 to 0.66 pg/g lipid. In 1994, EPA⁹ reported an average of 1.3 pg TEQ/g lipid for CDD/CDFs from grocery store grab samples of poultry reported in the literature. The national average CDD/CDF and PCB TEQ concentrations found in beef back fat, in a statistical survey designed analagously to the survey reported in this abstract^{2,3} (i.e., statistical sampling from federal slaughter establishments nationally, etc.), were 0.89 and 0.47 pg/g lipid, respectively. Similarly, the analagous national statistical survey for pork⁴ found national average CDD/CDF and PCB TEQ concentrations in pork belly fat to be 1.3 and 0.07 pg/g lipid, respectively. All concentrations reported in this paragraph were calculated assuming non-detected values are equal to 1/2 detection limit.

5. REFERENCES

- (1) L.S. Birnbaum; Cook, P.M.; Farland, W.; Preuss, P.; Schaum, J. *Organohalogen Compounds* 1993, 14, 1-4.
- (2) Winters, D.; Schaum, J. Organohalogen Compounds 1996, 30, 176-178.
- (3) Winters, D.; Cleverly, D.; Meier, K.; Dupuy, A.; Byrne, C.; Deyrup, C.; Ellis, R.; Ferrario, J.; Harless, R.; Leese, W.; Lorber, M.; McDaniel, D; Schaum, J.; Walcott, J. *Chemosphere* 1996, 32, 469-478.
- (4) Lorber, M.; Saunders, P.; Ferrario, J.; Leese, W.; Winters, D.; Cleverly, D.; Schaum, J.; Deyrup, C.; Ellis, R.; Walcott, J.; Dupuy, A.; Byrne, C.; McDaniel, D. *A Statistical Survey of Dioxin-Like Compounds in United States Pork Fat.* Presented at Dioxin '97 (this conference).
- (5) Ferrario, J.; Byrne, C.; McDaniel, D.; Dupuy, A. Analytical Chemistry 1995, 68, 647-652.
- (6) Ferrario, J.; Byrne, C.; Dupuy, A. Chemosphere 1997, 34, 2451-2465.
- (7) US EPA. Interim procedures for estimating risks associated with exposures to mixtures of chlorinated dibenzo--p-dioxins and -dibenzofurans (CDDS and CDFs) and the 1989 Update. Risk Assessment Forum, Washington, DC; EPA/625/3-89/016. 1989.
- (8) Ahlborg, U.G.; Becking, G.C.; Birnbaum, L.S.; Brouwer, A.; Derks, H.J.G.M; Feeley, M.;

- Golor, G.; Hanberg, A; Larsen, L.C.; Liem, A.K.D.; Safe, S.H.; Schlatter, C.; Waern, F.; Younes, M.; Yrjanheikki, E. *Chemosphere* 1994, 28, 1049-1067.
- (9) US EPA. Estimating Exposure to Dioxin-Like Compounds. Exposure Assessment Group, Office of Health and Environmental Assessment, Office of Research and Development. EPA/600/6-88/005Ca-c. Review Draft. June, 1994.
- (10) Stanley, J.S., Bauer, K.M. Chlorinated dibenzo-p-dioxin and dibenzofuran residue levels in food. Sacramento, CA: California Air Resources Board. 1989 ARB Contract No. A6-197-32.
- (11) A. Schecter, A.; Startin, J.; Wright, C.; Papke, O; Lis, A.; Ball, M. *Organohalogen Compounds* 1993, 13, 97-100.
- (12) Furst, P.; Furst, C.; Groebel, W. Chemosphere 1990, 20, 787-792.
- (13) Furst, P.; Furst, C.; Widmers, K. In: M. Gallo, R. Scheuplein, K. Van der Heijden, eds. Biological basis for risk assessment of dioxins and related compounds. Banbury Report #35. Plainview, NY, Cold Springs Harbor Laboratory Press 1991
- (14) Theelen, R.M.C., Liem, A.K.D., Slob, W.; Van Wijnen, J.H. *Chemosphere* 1993, 27, 1625-1635.
- (15) Taylor, J.K. *Quality Assurance of Chemical Measurements*. Chelsea, Michigan, Lewis Publishers, Inc., 1987.

Table 1. Overview of sampling frame (circa 1995/96), and number of samples from each category in the final survey (information from USDA's FSIS Animal Disposition Reporting System).

Animal Class	Number in survey	Percent of total slaughtered	
young chickens	41	7,412,000,000	94.4
light fowl	12	91,200,000	1.2
heavy fowl	12	60,800,000	0.8
young turkeys	15	282,000,000	3.6
TOTAL	80	7,846,200,000	100.0

Table 2. Comparison of CDD/CDF results of 2 high samples with remaining 78 samples of the national survey (results are in pg/g lipid; means and TEQ calculated at ND = 1/2 LOD and at ND = 0 in parenthesis; for maximum values, "ND" was given when there were no detections in the grouping; # pos = number of samples positive in group; all results pertain to raw survey data).

	Two High Samples	All O	ther Young Chi	ckens	Li	ght Fowl (n=12	2)	Heavy Fowl (n=12)			Young Turkeys (n=15)		
Congener	(Young Chickens)	# pos	mean	max	# pos	mean	max	# pos	mean	max	# pos	mean	max
2378-TCDD	16.82, 19.18	26	0.16 (0.15)	1.64	3	0.05 (0.03)	0.20	11	0.43 (0.42)	1.86	11	0.24 (0.24)	1.32
12378-PCDD	6.94, 9.35	8	0.24 (0.12)	1.38	0	0.15 (0)	ND	4	0.32 (0.22)	1.27	5	0.32 (0.23)	1.55
123478-HxCDD	0.86, 1.62	4	0.18 (0.05)	1.06	0	0.15 (0)	ND	3	0.24 (0.13)	0.81	2	0.16 (0.03)	0.32
123678-HxCDD	3.57, 5.68	24	0.39 (0.33)	3.17	8	0.34 (0.29)	0.69	11	0.71 (0.70)	2.35	13	0.79 (0.77)	2.02
123789-HxCDD	10.50, 12.36	12	0.39 (0.29)	3.87	1	0.15 (0.01)	0.15	5	0.60 (0.51)	3.09	3	0.17 (0.06)	0.39
1234678-HpCDD	9.98, 20.67	39	1.53 (1.53)	8.38	12	0.93 (0.93)	2.77	11	2.04 (2.02)	8.63	13	0.54 (0.52)	1.92
OCDD	47.83, 54.68	38	5.31 (5.31)	41.03	12	2.07 (2.07)	6.43	12	7.67 (7.67)	20.26	11	0.75 (0.68)	2.40
2378-TCDF	0.19, 0.24	35	0.28 (0.28)	1.59	9	0.25 (0.25)	1.68	11	0.48 (0.47)	1.40	12	0.57 (0.57)	2.57
12378-PCDF	0.16(0), 0.15(0)	5	0.21 (0.08)	1.04	1	0.18 (0.05)	0.56	2	0.14 (0.02)	0.15	4	0.36 (0.25)	2.56
23478-PCDF	0.16 (0), 0.15 (0)	5	0.25 (0.12)	1.82	3	0.22 (0.11)	0.84	4	0.18 (0.09)	0.32	9	0.53 (0.47)	3.83
123478-HxCDF	0.16 (0), 0.15 (0)	5	0.23 (0.10)	1.91	2	0.16 (0.04)	0.25	3	0.17 (0.06)	0.32	8	0.20 (0.13)	0.76
123678-HxCDF	0.16 (0), 0.15 (0)	5	0.20 (0.07)	1.61	2	0.15 (0.03)	0.21	1	0.15 (0.01)	0.18	1	0.17 (0.03)	0.52
123789-HxCDF	0.16 (0), 0.15 (0)	0	0.15 (0)	ND	0	0.15 (0)	ND	0	0.15 (0)	ND	0	0.15 (0)	ND
234678-HxCDF	0.16 (0), 0.15 (0)	6	0.21 (0.08)	2.21	2	0.14 (0.02)	0.13	2	0.15 (0.02)	0.21	2	0.15 (0.03)	0.25
1234678-HpCDF	0.16 (0), 0.84	19	0.27 (0.20)	2.72	4	0.15 (0.05)	0.26	4	0.20 (0.10)	0.62	2	0.15 (0.02)	0.27
1234789-HpCDF	0.16 (0), 0.15 (0)	4	0.17 (0.04)	0.69	0	0.15 (0)	ND	0	0.15 (0)	ND	0	0.15 (0)	ND
OCDF	0.31 (0), 0.55	5	0.34 (0.07)	1.08	0	0.29(0)	ND	1	0.31 (0.04)	0.44	0	0.29 (0)	ND
CDD/CDF TEQ	22 (22), 26 (26)		0.64 (0.41)	3.4		0.40 (0.16)	0.92		0.98 (0.80)	2.33		0.93 (0.76)	3.94

Table 3. Comparison of coplanar PCB results of 2 high samples with remaining 78 samples of the national survey (all results are in pg/g lipid; means

and TEQ calculated at ND = 1/2 LOD and at ND = 0 in parenthesis; # pos = number of samples positive in group; all results pertain to raw survey data).

Congener	Two High Samples	All O	All Other Young Chickens (n=39)			Light Fowl (n=12)			Heavy Fowl (n=12)			Young Turkeys (n=15)		
	(Young Chickens)	# pos	mean	max	# pos	mean	max	# pos	mean	max	# pos	mean	max	
PCB 77	4.3, 5.0	39	9.3	46.3	12	12.2	50.3	12	10.6	25.6	12	5.6	19.8	
PCB 118	214, 296	39	522	3995	12	599	1687	12	663	2454	12	1116	2711	
PCB 105	70, 85	39	132	549	12	171	423	12	165	452	12	307	597	
PCB 126	1.1, 2.0	39	1.8	10.8	12	1.6	4.5	12	2.2	6.4	12	4.4	9.0	
PCB 156	21, 31	39	41	199	11	58	168	12	54	148	12	108	198	
PCB 157	6.4, 7.5	39	10.5	54.6	11	12.5 (12.6)	36.8	12	13.3	40.9	12	26.2	51.2	
PCB 169	0.05 (0), 0.3	31	0.2 (0.2)	1.2	8	0.2 (0.2)	0.4	11	0.4 (0.4)	0.8	12	0.6	1.1	
PCB TEQ	0.15, 0.26		0.28 (0.28)	1.7		0.28 (0.28)	0.77		0.34 (0.34)	1.05		0.66	1.29	